Supplemental Information

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Resolution of Gene Regulatory Conflicts Caused by Combinations of Antibiotics

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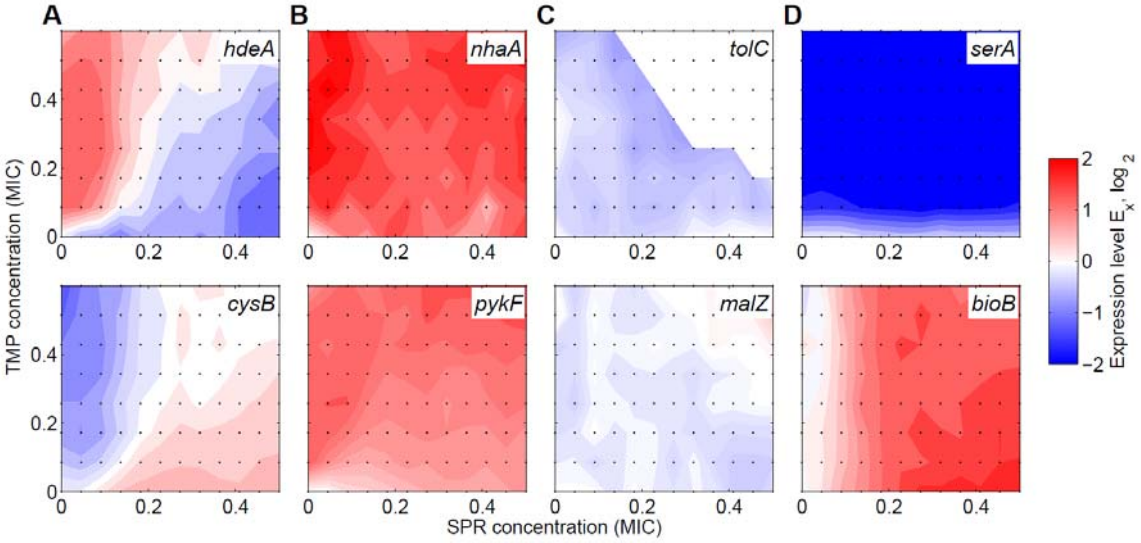

Figure S1. Examples of Different Types of Gene Expression Responses in Two-Dimensional Concentration Gradients of TMP and SPR

Expression level E_x is shown in color code: blue indicates down-regulation, red up-regulation, and white no change in gene expression (*cf.* Figure 3B). **(A)** The promoters *hdeA* and *cysB* both show conflicting responses, i.e. they are oppositely regulated in response to the individual drugs TMP and SPR. **(B)** The promoters *nhaA* and *pykF* both show consistent responses, i.e. they are up-regulated in response to both individual drugs. **(C)** The promoters *tolC* and *malZ* show no or very weak responses to these drugs. **(D)** The promoters *serA* and *bioB* respond almost exclusively to TMP and almost exclusively to SPR, respectively. In these examples, the expression level in the drug combination mostly lies between the levels in the individual drugs as is the case for most promoters (see also Figure 3B). Drug concentrations are in units of the Minimal Inhibitory Concentration (MIC, see Table 1).

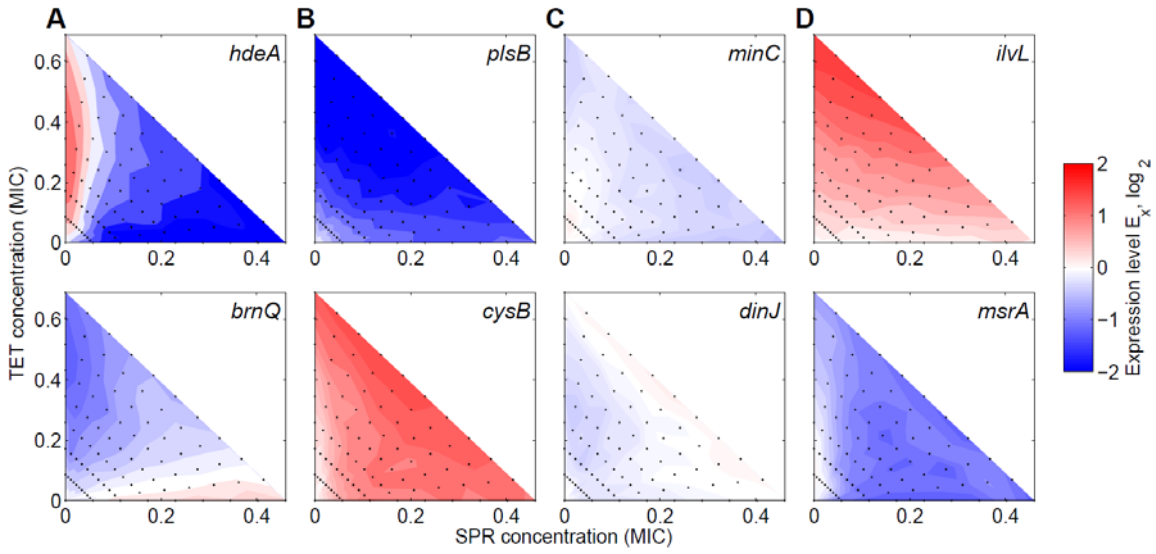

Figure S2. Examples of Different Types of Gene Expression Responses in Two-Dimensional Concentration Gradients of TET and SPR

Expression level E_x is shown in color code (*cf.* Figures 3B, S1). **(A)** The promoters *hdeA* and *brnQ* both show conflicting responses. **(B)** The promoters *plsB* and *cysB* both show consistent responses. **(C)** The promoters *minC* and *dinJ* show no or very weak responses to the drugs TET and SPR alone or in combination. **(D)** The promoters *ilvL* and *msrA* respond almost exclusively to TET and almost exclusively to SPR, respectively. In these examples, the expression level in the drug combination lies between the levels in the individual drugs which is the case for most promoters (see also Figures 3B, S1). Drug concentrations are in units of the MIC (see Table 1).

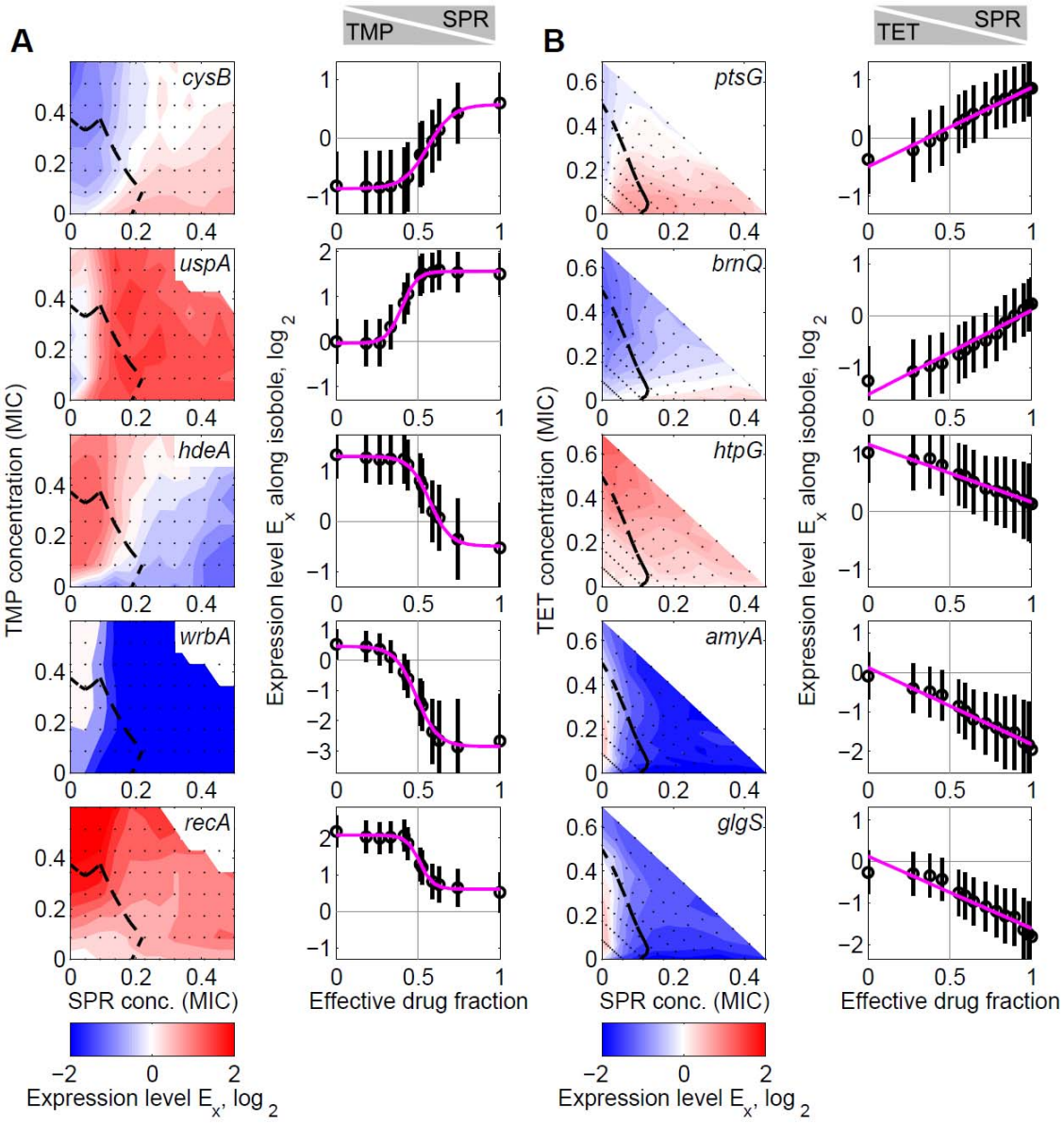

Figure S3. Examples of Promoters that Resolve Gene Regulatory Conflicts by Prioritizing in the TMP-SPR Drug Combination or by Averaging in the TET-SPR Combination

(A) Expression levels of promoters *cysB*, *uspA*, *hdeA*, *wrbA*, and *recA* in two-dimensional drug concentration space of TMP-SPR in color code (see also Figure 5C). Gene expression levels along the growth rate isobole (dashed black line, normalized growth rate g=0.5) are shown on the right. Magenta lines:

sigmoidal fits (see Experimental Procedures). Conflicts in gene expression are resolved in a prioritized response, leading to a relatively sharp transition between the conflicting expression levels as TMP is continuously replaced with SPR (*cf.* Figure 1B). **(B)** As A, but for different example genes *ptsG*, *brnQ*, *htpG*, *amyA,* and *glgS*, which show conflicts in the two-dimensional drug concentration space of TET-SPR (see also Figure 5D). Magenta lines: linear fits (see Experimental Procedures).Conflicts in gene expression are smoothly averaged, leading to a linear transition between the conflicting expression levels (*cf.* Figure 1B). Error-bars correspond to two standard deviations estimated from replicate measurements done on different days (Experimental Procedures).

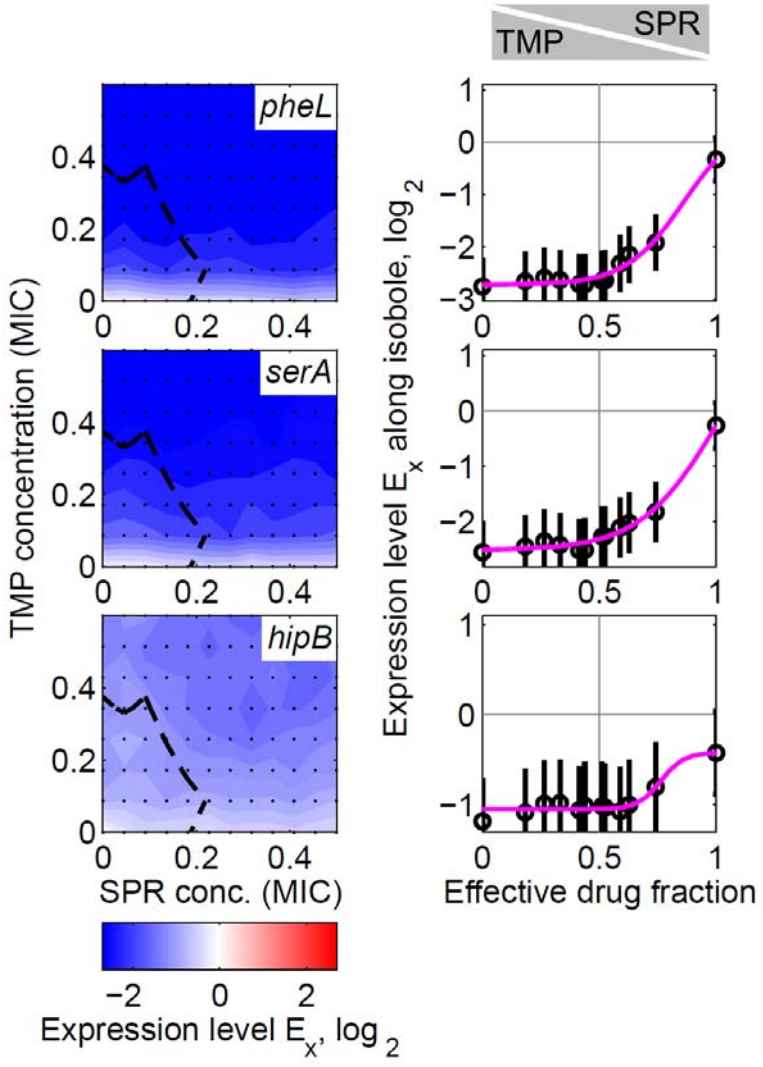

Figure S4. Examples of Promoters Showing a Biased Prioritized Response to the TMP-SPR Drug Combination

Expression levels of promoters *pheL*, *serA*, and *hipB* in two-dimensional drug concentration space of TMP-SPR in color code. Gene expression levels along the growth rate isobole (dashed black line, normalized growth rate g=0.5) are shown on the right. Magenta lines: sigmoidal fits (see Experimental Procedures). In the TMP-SPR drug combination, these promoters show a biased prioritized response (*cf.* Figure 1B), i.e. the transition point x_0 of the prioritized response

(inset Figure 5E) is not located at effective drug fraction 0.5 where both drugs contribute equally to growth inhibition. The promoters shown here are all dominated by TMP, i.e. the transition point is located near the pure SPR drug environment. Error-bars correspond to two standard deviations estimated from replicate measurements done on different days (Experimental Procedures).

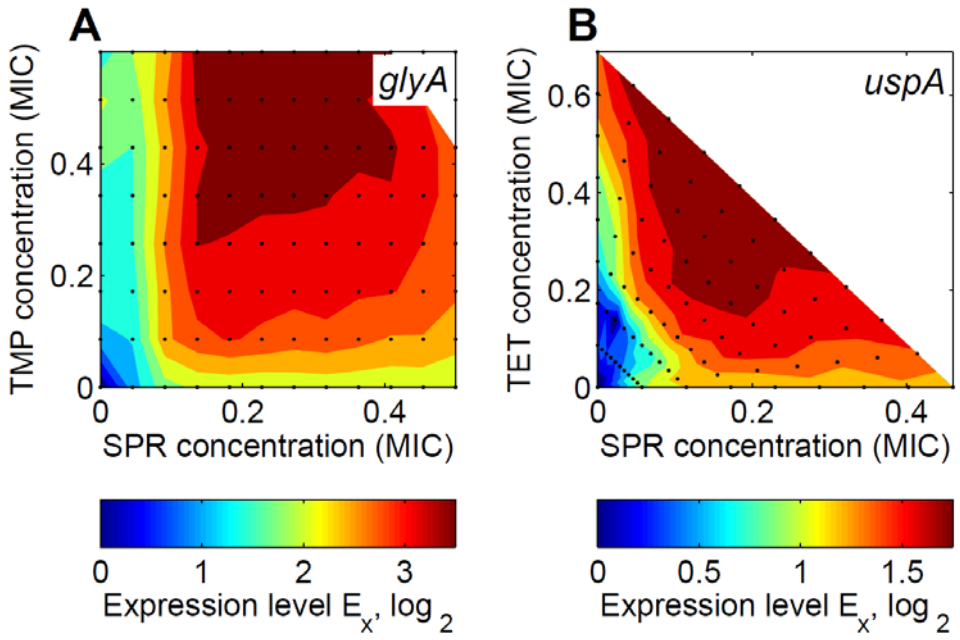

Figure S5. Examples of Promoters Showing Combination-Specific Responses to Antibiotic Combinations

(A) Expression levels of promoter *glyA* in two-dimensional drug concentration space of TMP-SPR in color code. The *glyA* promoter, which has a large score for the third principal component (Figure 6A), is up-regulated in response to TMP and SPR individually and up-regulated to much higher levels in the combination. Similar to *slp* (Figure 6D), *glyA* expression is regulated by multiple transcription factors (PurR and MetR). **(B)** The *uspA* promoter shows a similar drug combination-specific response to the TET-SPR combination. However, *uspA* is the only promoter in our data set (Table S1) showing this behavior for the TET-SPR combination. Similar to *glyA* (A) and *slp* (Figure 6D), *uspA* is known to be regulated by two different transcription factors (IHF and FadR).

Table S1. Genome-Wide Sample of 103 Promoters Used in This Study

Promoter	Description
amyA	cytoplasmic alpha-amylase
*arcA**	response regulator (OmpR family), in two-component regulatory system with ArcB (or CpxA), regulates genes in aerobic respiration (1st module)
aroH	3-deoxy-D-arabinoheptulosonate-7-phosphate synthase (DAHP synthetase), tryptophan repressible
aroL♦	shikimate kinase II
atpI	membrane-bound ATP synthase subunit, F1-F0-type proton-ATPase
bioB	biotin synthetase (2nd module)
bolA	activator of morphogenic pathway (BolA family), important in general stress response
brnQ	LIVCS family, branched chain amino acid transporter system II (LIV-II)
clpP	proteolytic subunit of clpA-clpP ATP-dependent serine protease, heat shock protein F21.5
crp	transcriptional regulator, catabolite activator protein (CAP), cyclic AMP receptor protein (CAMP-binding family), interacts with RNAP
cspA#	major cold shock protein 7.4, transcription antiterminator of hns,
cspD	similar to CspA but not cold shock induced, nucleic acid-binding domain
cyoA	cytochrome o ubiquinol oxidase subunit II
cysB	transcriptional regulator for biosynthesis of L-cysteine (LysR familiy) (1st module)
cysP	ABC superfamily (peri_bind) thiosulfate transport protein
dacA	D-alanyl-D-alanine carboxypeptidase, penicillin-binding protein 5 (1st module)
dinJ	damage-inducible protein J
dinP♦	DNA polymerase IV, devoid of proofreading, damage-inducible protein P (1st module)
*dnaK**	chaperone Hsp70 in DNA biosynthesis/cell division (1st module)
dnaX	DNA polymerase III, tau and gamma subunits; DNA elongation factor III (1st module)
dps#	stress response DNA-binding protein; starvation induced resistance to H2O2, ferritin-like
*emrE**	DLP12 prophage; MFP family auxillary multidrug transport protein, methylviologen and ethidium resistance
evgA	response regulator (activator) in two-component regulatory system with EvgS, regulates multidrug resistance (LuxR/UhpA family)
fecI	sigma (19) factor of RNA polymerase, affected by FecR and outer membrane receptor FecA (TetR/ArcR family)
fepA	outer membrane porin, receptor for ferric enterobactin (enterochelin) and colicins B and D (1st module)
*fliA**♦	sigma F (sigma 28) factor of RNA polymerase, transcription of late flagellar genes (class 3a and 3b operons)
folA	dihydrofolate reductase type I; trimethoprim resistance
*fumC**♦	fumarase C (fumarate hydratase Class II) (2nd module)
galE	UDP-galactose 4-epimerase (1st module)
glgS	glycogen biosynthesis, rpoS dependent
glyA#	serine hydroxymethyltransferase (2nd module)
gnd	gluconate-6-phosphate dehydrogenase, decarboxylating (1st module)
hdeA	HdeA dimer, inactive form of acid-resistance protein
hipB	transcriptional repressor which interacts with HipA
hisL	his operon leader peptide
hisS	histidine tRNA synthetase (operon includes yfgL, see D. Kahne, Science, 2001)
htpG	chaperone Hsp90, heat shock protein C 62.5
htpX	heat shock protein, integral membrane protein

icdA	isocitrate dehydrogenase in e14 prophage, specific for NADP+ (2nd module)
ileX[#]	isoleucine tRNA 2
ilvL	ilvGEDA operon leader peptide
inaA[♦]	pH inducible protein involved in stress response, protein kinase-like
lexA	transcriptional repressor for SOS response (signal peptidase of LexA family)
lpdA	dihydrolipoamide dehydrogenase, FAD/NAD(P)-binding ; component of 2-oxodehydrogenase and pyruvate complexes; L protein of glycine cleavage complex second part (2nd module)
malZ[♦]	maltodextrin glucosidase (2nd module)
*marR**[♦]	transcriptional repressor for antibiotic resistance and oxidative stress
minC	cell division inhibitor; activated MinC inhibits FtsZ ring formation
mscL[♦]	mechanosensitive channel
msrA	peptide methionine sulfoxide reductase
*nfo**	endonuclease IV
*nhaA**	NhaA family of transport protein, Na+/H antiporter (1st module)
*nuoA**	NADH dehydrogenase I chain A
osmC	resistance protein, osmotically inducible
*oxyR**	transcriptional regulator of oxidative stress, regulates intracellular hydrogen peroxide (LysR family)
pheL	leader peptide of chorismate mutase-P-prephenate dehydratase
plsB	glycerolphosphate acyltransferase (2nd module)
polB[♦]	DNA polymerase II and and 3' --> 5' exonuclease
*poxB**[♦]	pyruvate dehydrogenase/oxidase FAD and thiamine PPi binding, cytoplasmic in absence of cofactors (1st module)
ptsG	multimodular PtsG: PTS family enzyme IIC, glucose-specific (1st module)
pykF	pyruvate kinase I (formerly F), fructose stimulated (2nd module)
recA[#]	DNA strand exchange and recombination protein with proteiase and nuclease activity (1st module)
recN[♦]	protein used in recombination and DNA repair (2nd module)
rmf[#]	ribosome modulation factor (involved in dimerization of 70S ribosomes)
rob	(activator of acrAB operon) transcriptional activator for resistance to antibiotics, organic solvents and heavy metals (AraC/XylS family) (right origin binding protein) (1st module)
rpiA	ribosephosphate isomerase, constitutive
rplN	50S ribosomal subunit protein L14
rplY	50S ribosomal subunit protein L25
rpmB	50S ribosomal subunit protein L28
rpmE[#]	50S ribosomal subunit protein L31
*rpoD**[♦]	sigma D (sigma 70) factor of RNA polymerase , major sigma factor during exponential growth (2nd module)
rpoH	sigma H (sigma 32) factor of RNA polymerase; transcription of heat shock proteins induced by cytoplasmic stress
*rpoS**[♦]	sigma S (sigma 38) factor of RNA polymerase, major sigmafactor during stationary phase
rpsA	30S ribosomal subunit protein S1 (3rd module)
rpsB	30S ribosomal subunit protein S2
*rpsT**	30S ribosomal subunit protein S20
rpsU	30S ribosomal subunit protein S21
rrsA[#]	16S rRNA
rsd	regulator of sigma D, has binding activity to the major sigma subunit of RNAP

sbmC♦	DNA gyrase inhibitor
sdhC	succinate dehydrogenase , cytochrome b556
serA	D-3-phosphoglycerate dehydrogenase
serC	3-phosphoserine aminotransferase / phosphohydroxythreonine transaminase
serU	serine tRNA 2
slp#	outer membrane protein, induced after carbon starvation
smpA	small membrane protein A
*sodA**	superoxide dismutase, manganese
sspA	stringent starvation protein A, regulator of transcription
thiC	5'-phosphoryl-5-aminoimidazole = 4-amino-5-hydroxymethyl-2-methylpyrimidine-P
*tolA**♦	tol protein required for outer membrane integrity, uptake of group A colicins, C-terminal is coreceptor with F pilus for filamentous phages, role in translocation of filamenous phage DNA to cytoplasm (1st module)
*tolC**	outer membrane channel; specific tolerance to colicin E1; segregation of daughter chromosomes, role in organic solvent tolerance
*trpR**	transcriptional repressor for tryptophan biosynthesis (TrpR family)
*umuD**♦	component of DNA polymerase V , signal peptidase with UmuC
uspA	universal stress protein A
uvrA♦	UvrA with UvrBC is a DNA excision repair enzyme (2nd module)
*uvrD**	DNA-dependent ATPase I and helicase II (1st module)
wrbA#	flavodoxin-like protein, trp repressor binding protein
*yaeL**♦	putative protease
*ydeB**	marC, inner membrane protein involved in multiple antibiotic resistance
*ydhE**	NorE multidrug efflux MATE transporter (1st module)
*yebG**♦	DNA damage-inducible gene in SOS regulon, dependent on cyclic AMP and H-NS
*yebQ**	putative MFS family transport protein (1st module)
yojH	
yojI	putative ABC superfamily (atp module of atp&membrane) transport protein (2nd module)

♦ Promoters with low GFP signal. These were excluded from further analysis.

* Expression of these promoters was measured only in TMP-SPR to test if the PCA results change when the set of promoters is enlarged. All results, in particular the PCA results in Figures 4-6, are independent of whether these promoters are included in the analysis or not. To compare PCA results of the TMP-SPR and TET-SPR data sets, these promoters were excluded from the analysis, so that the exact same set of promoters is used for both drug pairs.

These promoters were integrated in the chromosome for the single-cell experiment (Figure 7).